	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	2017	codon same optimiz$5	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:00			0
2	BRS	L2	5901	human adj protein	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:00			0
3	BRS	L3	661	(human adj factor adj VIII) or (human adj factor adj IX)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:01			0
4	BRS	L4	1	1 same (2 or 3)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:02			0
5	BRS	L5	0	(common adj codon) same (non$1common adj codon)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:04			0
6	BRS	L6	22	(common adj codon)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:04			0
7	BRS	L7	0	(non-common adj codon)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:04			0
8	BRS	L8	0	(non adj common adj codon)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:05			0
9	BRS	L9	0	(less adj common adj codon)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:05			0
10	BRS	L10	1	6 same (2 or 3)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:05			0

	Type	L #	Hits	Search Text	DBs	Time Stamp
1	BRS	L1	65	codon adj optimization	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:49
2	BRS	L2	4298	expression same human same (mammalian adj cell)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:50
3	BRS	L3	1	1 same 2	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/08/14 14:50

> d his

(FILE 'HOME' ENTERED AT 14:33:44 ON 14 AUG 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
14:34:21 ON 14 AUG 2002

L1 118 S CODON OPTIMIZATION
L2 21610 S HUMAN PROTEIN
L3 5327 S (HUMAN FACTOR IX) OR (HUMAN FACTOR VIII)
L4 359 S CODON (A) OPTIMIZ?
L5 3 S (L1 OR L4) (P) (L2 OR L3)
L6 1 DUPLICATE REMOVE L5 (2 DUPLICATES REMOVED)
L7 9675 S EXPRESSION (P) HUMAN (P) (MAMMALIAN CELL)
L8 32 S L7 (P) (L1 OR L4)
L9 8 DUPLICATE REMOVE L8 (24 DUPLICATES REMOVED)

=> log y

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FILE 'HOME' ENTERED AT 14:33:44 ON 14 AUG 2002

=> file medline caplus biosis embase scisearch agricola
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 14:34:21 ON 14 AUG 2002

FILE 'CAPLUS' ENTERED AT 14:34:21 ON 14 AUG 2002
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FILE 'SCISEARCH' ENTERED AT 14:34:21 ON 14 AUG 2002
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FILE 'AGRICOLA' ENTERED AT 14:34:21 ON 14 AUG 2002

=> s codon optimization
L1          118 CODON OPTIMIZATION

=> s human protein
   4 FILES SEARCHED...
L2        21610 HUMAN PROTEIN

=> s (human factor IX) or (human factor VIII)
   3 FILES SEARCHED...
L3         5327 (HUMAN FACTOR IX) OR (HUMAN FACTOR VIII)

=> s codon (a) optimiz?
L4          359 CODON (A) OPTIMIZ?

=> s (l1 or l4) (p) (l2 or l3)
L5            3 (L1 OR L4) (P) (L2 OR L3)

=> duplicate remove l5
DUPLICATE PREFERENCE IS 'MEDLINE, CAPLUS, SCISEARCH'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L5
L6             1 DUPLICATE REMOVE L5 (2 DUPLICATES REMOVED)

=> d l6 1 ibib abs

L6   ANSWER 1 OF 1     MEDLINE                              DUPLICATE 1
ACCESSION NUMBER:   2000494820     MEDLINE
DOCUMENT NUMBER:    20442636   PubMed ID: 10985959
TITLE:              Fusion protein vectors to increase protein production and
                    evaluate the immunogenicity of genetic vaccines.
AUTHOR:             Wu L; Barry M A
CORPORATE SOURCE:   Center for Cell and Gene Therapy, Baylor College of
                    Medicine, Houston, Texas, 77030, USA.
CONTRACT NUMBER:    AI042588 (NIAID)
     AI36211 (NIAID)
SOURCE:             MOLECULAR THERAPY, (2000 Sep) 2 (3) 288-97.
                    Journal code: 100890581. ISSN: 1525-0016.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals; AIDS
ENTRY MONTH:        200010
ENTRY DATE:         Entered STN: 20001027
                    Last Updated on STN: 20001027
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Entered Medline: 20001013

AB Genetic immunization is a method for vaccination and laboratory antibody production where antigen-expressing plasmids are introduced into animals to elicit immune responses. Although genetic immunization works well for many antigens, problems can arise with protein sequences that (i) are toxic to host cells, (ii) are difficult to translate by mammalian cells, or (iii) evade immune presentation. We demonstrate here the ability to increase protein production and antigen secretion by the simple method of fusing poorly expressed sequences to well-expressed heterologous proteins. Proof-of-principle is demonstrated here using the poorly translated HIV-1 envelope whose protein production is rescued by fusing this antigen to the carboxy-termini of two well-expressed proteins: the cytoplasmic green fluorescent protein and the secreted ***human*** ***protein*** a1-antitrypsin. This approach represents a simple and substantially less expensive method to increase protein and antigen production than ***codon*** - ***optimization*** strategies. It may therefore be more useful than whole gene codon replacement to enable inexpensive laboratory antibody production of poorly expressed antigens and for large-scale genomic protein or antigen screening efforts. Finally, we demonstrate a second benefit of this antigen fusion strategy in which the test antigen is "sandwiched" between two positive control antigens. By this approach, we demonstrate the intrinsic lack of immunogenicity of HIV-1 envelope under conditions when robust antibody responses are generated against its fusion protein partners, but not against this evasive antigen. These fusion protein vectors therefore represent a simple approach to not only increase antigen production, but also assess antigen production and immunogenicity in vivo.

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=> d his

     (FILE 'HOME' ENTERED AT 14:33:44 ON 14 AUG 2002)

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
     14:34:21 ON 14 AUG 2002
L1           118 S CODON OPTIMIZATION
L2         21610 S HUMAN PROTEIN
L3          5327 S (HUMAN FACTOR IX) OR (HUMAN FACTOR VIII)
L4           359 S CODON (A) OPTIMIZ?
L5             3 S (L1 OR L4) (P) (L2 OR L3)
L6             1 DUPLICATE REMOVE L5 (2 DUPLICATES REMOVED)

=> s expression (p) human (p) (mammalian cell)
   3 FILES SEARCHED...
L7          9675 EXPRESSION (P) HUMAN (P) (MAMMALIAN CELL)

=> s l7 (p) (l1 or l4)
L8            32 L7 (P) (L1 OR L4)

=> duplicate remove l8
DUPLICATE PREFERENCE IS 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L8
L9             8 DUPLICATE REMOVE L8 (24 DUPLICATES REMOVED)

=> d l9 1-8 ibib abs

L9   ANSWER 1 OF 8  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2001:923841  CAPLUS
DOCUMENT NUMBER:          136:49344
TITLE:                    Codon optimized recombinant Dermatophagoides allergen
                          ProDer p I, recombinant production, purification and
                          IgE and IgG-reactivities
INVENTOR(S):              Bollen, Alex; Jacobs, Paul; Jacquet, Alain; Massaer,
                          Marc Georges Francis
PATENT ASSIGNEE(S):       Smithkline Beecham Biologicals S.A., Belg.
SOURCE:                   PCT Int. Appl., 48 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:
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PATENT NO. KIND DATE APPLICATION NO. DATE

WO 2001096385 A1 20011220 WO 2001-EP6483 20010607

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: GB 2000-14288 A 20000610

AB The present invention relates to ***codon*** ***optimized*** polynucleotides which are efficiently expressed in ***mammalian*** ***cells*** and encode insect proteins from Dermatophagoides dust mite. In particular, the ***optimized*** ***codon*** polynucleotides encode a protein from Dermatophagoides pteronyssinus, such as Der P I or proDer P I. The codons are selected from highly expressed ***human*** gene and humanized ProDer p I gene was generated. The native Der p I signal sequence was exchanged with the highly efficient leader peptide of VZV glycoprotein E (gE) to facilitate secretion. The present invention also provides methods of prepg. pharmaceutical compns. comprising the ***expression*** of the ***codon*** ***optimized*** polynucleotides, and vectors and transformed host cells comprising them. The invention demonstrated that recombinant ProDer P I display reactivities similar to those of native Der P I towards specific anti-Der P I IgG and anti-Dermatophagoides pteronyssinus IgE, suggesting that recProDer P I displayed the overall structure of the natural allergen. The invention provides a method for efficient ***expression*** of recombinant Dermatophagoides allergens for use in the manuf. of pharmaceuticals, vaccine or diagnostic assays.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 8 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2001555989 MEDLINE

DOCUMENT NUMBER: 21488669 PubMed ID: 11602739

TITLE: Multiple effects of codon usage optimization on expression and immunogenicity of DNA candidate vaccines encoding the human immunodeficiency virus type 1 Gag protein.

AUTHOR: Deml L; Bojak A; Steck S; Graf M; Wild J; Schirmbeck R; Wolf H; Wagner R

CORPORATE SOURCE: Institute of Medical Microbiology, University of Regensburg, 93053 Regensburg, Germany.

SOURCE: JOURNAL OF VIROLOGY, (2001 Nov) 75 (22) 10991-1001. Journal code: 0113724. ISSN: 0022-538X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200112

ENTRY DATE: Entered STN: 20011017
Last Updated on STN: 20020122
Entered Medline: 20011204

AB We have analyzed the influence of codon usage modifications on the ***expression*** levels and immunogenicity of DNA vaccines, encoding the ***human*** immunodeficiency virus type 1 (HIV-1) group-specific antigen (Gag). In the presence of Rev, an ***expression*** vector containing the wild-type (wt) gag gene flanked by essential cis-acting sites such as the 5'-untranslated region and 3'-Rev response element supported substantial Gag protein ***expression*** and secretion in ***human*** H1299 and monkey COS-7 cells. However, only weak Gag production was observed from the murine muscle cell line C2C12. In contrast, optimization of the Gag coding sequence to that of highly expressed mammalian genes (syngag) resulted in an obvious increase in the G+C content and a Rev-independent ***expression*** and secretion of Gag in all tested ***mammalian*** ***cell*** lines, including murine C2C12 muscle cells. Mice immunized intramuscularly with the syngag plasmid showed Th1-driven humoral and cellular responses that were substantially higher than those obtained after injection of the

Rev-dependent wild-type (wt) gag vector system. In contrast, intradermal immunization of both wt gag and syngag vector systems with the particle gun induced a Th2-biased antibody response and no cytotoxic T lymphocytes. Deletion analysis demonstrated that the CpG motifs generated within syngag by ***codon*** ***optimization*** do not contribute significantly to the high immunogenicity of the syngag plasmid. Moreover, low doses of coadministered stimulatory phosphorothioate oligodeoxynucleotides (ODNs) had only a weak effect on antibody production, whereas at higher doses immunostimulatory and nonstimulatory ODNs showed a dose-dependent suppression of humoral responses. These results suggest that increased Gag ***expression*** , rather than modulation of CpG-driven vector immunity, is responsible for the enhanced immunogenicity of the syngag DNA vaccine.

L9 ANSWER 3 OF 8 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001699199 MEDLINE
DOCUMENT NUMBER: 21614869 PubMed ID: 11747598
TITLE: Helper plasmids for production of HIV-1-derived vectors.
AUTHOR: Fuller M; Anson D S
CORPORATE SOURCE: Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide South Australia, 5006.
SOURCE: HUMAN GENE THERAPY, (2001 Nov 20) 12 (17) 2081-93. Journal code: 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011219
Last Updated on STN: 20020208
Entered Medline: 20020207

AB Vectors derived from ***human*** immunodeficiency virus type 1 (HIV-1) appear an attractive option for many gene therapy applications. This is due to their ability to transduce noncycling cell populations and to integrate their genome into the host cell chromosome, resulting in the stable genetic modification of the transduced cell. These properties have permitted the direct in vivo transduction of several tissues, including the central nervous system, retina, and liver. However, the pathogenic nature of HIV-1 has raised considerable concerns about the safety of such vector systems. To help address these concerns, we have expressed each of the primary transcriptional units encoding trans functions relevant for vector production in individual plasmid constructs. The gag-pol gene sequence was ***codon*** - ***optimized*** for ***expression*** in ***mammalian*** ***cells*** resulting in high level Rev/Rev-response element (RRE)-independent ***expression*** . ***Codon*** ***optimization*** of gag-pol also reduces sequence homology with vectors containing gag gene sequences, which results in reduced transfer of biologically active gag-pol sequences to transduced cells. Furthermore, the vif reading frame overlapping the 3' end of the pol coding sequence is destroyed by ***codon*** ***optimization*** . We have also shown that the Gag and Gag-Pol polyproteins can be efficiently expressed from separate transcriptional units. This has enabled the removal of a cis-acting viral element, the gag-pol translational frameshift sequence, from the vector/packaging system and prevents detectable transfer of biologically active sequences equivalent to the gag-pol gene to transduced cells.

L9 ANSWER 4 OF 8 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001191455 MEDLINE
DOCUMENT NUMBER: 21093141 PubMed ID: 11177542
TITLE: Retroviral expression of Escherichia coli thymidylate synthase cDNA confers high-level antifolate resistance to hematopoietic cells.
AUTHOR: Shaw D; Berger F G; Spencer H T
CORPORATE SOURCE: Department of Biological Sciences and the South Carolina Cancer Center University of South Carolina, Columbia, SC 29208, USA.
CONTRACT NUMBER: CA 78651 (NCI)
CA4401 (NCI)
SOURCE: HUMAN GENE THERAPY, (2001 Jan 1) 12 (1) 51-9. Journal code: 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010410
Last Updated on STN: 20010410
Entered Medline: 20010405

AB Drug resistance gene therapy has the potential to protect against the myelosuppressive side effects of chemotherapy or to be used as a dominant in vivo selectable marker of genetically modified cells. Steady state kinetic studies have indicated the Escherichia coli thymidylate synthase (ecTS) is intrinsically more resistant to several TS-directed inhibitors as compared with the ***human*** enzyme, suggesting that ecTS is suitable for use as a drug-resistant marker. However, we found a disparity between the kinetic properties of ecTS and the degree of resistance conferred to cells transfected with the cDNA encoding this enzyme. It was determined that although ecTS is as stable as ***human*** TS (hTS) in transfected ***mammalian*** ***cells*** , ecTS is produced at only 40% the level of hTS, indicating poor translation of ecTS in eukaryotic cells. To circumvent this problem, the entire cDNA sequence of ecTS was synthesized by using ***codons*** ***optimized*** for ***expression*** in ***mammalian*** ***cells*** . In transfected Chinese hamster lung cells, ***expression*** of ecTS from the optimized construct, termed OPTecTS, is as efficient as hTS. Furthermore, cells transfected with the OPTecTS cDNA are significantly more resistant to the TS inhibitor raltitrexed as compared with transfected cells expressing similar levels of hTS. High-titer retroviral packaging cells were generated with OPTecTS and >80% of transduced mouse hematopoietic progenitor cells are resistant to raltitrexed, Thymitaq, and U89 at concentrations that eliminated colony growth of mock-transduced cells. The transgene was detectable by PCR in transduced bone marrow selected in U89 or raltitrexed, and ***expression*** of ecTS from the OPTecTS cDNA in bone marrow exhibited a catalytic rate constant comparable to that of purified recombinant ecTS. These data indicate that OPTecTS is a viable dominant selectable marker that can confer resistance to antifolates when introduced into cells.

L9 ANSWER 5 OF 8 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001311896 MEDLINE
DOCUMENT NUMBER: 21278642 PubMed ID: 11385286
TITLE: High-level expression in mammalian cells of recombinant house dust mite allergen ProDer p 1 with optimized codon usage.
AUTHOR: Massaer M; Mazzu P; Haumont M; Magi M; Daminet V; Bollen A; Jacquet A
CORPORATE SOURCE: Department of Applied Genetics, Institut de Biologie et de Medecine Moleculaires, Universite Libre de Bruxelles, Gosselies, Belgium.
SOURCE: INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2001 May) 125 (1) 32-43.
Journal code: 9211652. ISSN: 1018-2438.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB BACKGROUND: The major house dust mite allergen Der p 1 is associated with allergic diseases such as asthma. Production of recombinant Der p 1 was previously attempted, but with limited success. The present study describes the ***expression*** of recombinant (rec) ProDer p 1, a recombinant precursor form of Der p 1, in CHO cells. METHODS: As optimization of the codon usage may allow successful overexpression of protein in ***mammalian*** ***cells*** , a synthetic gene encoding ProDer p 1 was designed on the basis of the codon usage frequently found in highly expressed ***human*** genes. Gene synthesis was accomplished from a set of 14 mutually priming overlapping oligonucleotides and after two runs of polymerase chain reaction. RESULTS: COS cells transiently transfected with the synthetic ProDer p 1 gene produced up to 5--10 times as much ProDer p 1 compared with the ***expression*** level obtained after transfection with the authentic gene. To stably express the

recombinant allergen, CHO-K1 cells were transfected with the ProDer p 1 synthetic gene, and one amplified recombinant clone produced up to 30 mg of recProDer p 1 per liter in the culture medium before purification. recProDer p 1 was secreted as an enzymatically inactive single-chain molecule presenting three glycosylated immunoreactive forms of 41, 38 and 36 kD. When examined with respect to direct binding, recProDer p 1 and natural Der p 1 displayed very similar IgE reactivities. However, IgE inhibition and histamine release assays showed a much higher reactivity to natural Der p 1 compared to recProDer p 1. CONCLUSIONS: These data indicated that ***codon*** ***optimization*** represents an attractive strategy for high-level production of allergen in ***mammalian*** ***cells*** .

L9 ANSWER 6 OF 8 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999428556 MEDLINE
DOCUMENT NUMBER: 99428556 PubMed ID: 10497246
TITLE: Enhanced expression, native purification, and characterization of CCR5, a principal HIV-1 coreceptor.
AUTHOR: Mirzabekov T; Bannert N; Farzan M; Hofmann W; Kolchinsky P; Wu L; Wyatt R; Sodroski J
CORPORATE SOURCE: Department of Cancer Immunology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.
CONTRACT NUMBER: AI41851 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Oct 1) 274 (40) 28745-50.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991102

AB Seven-transmembrane segment, G protein-coupled receptors (GPCRs) play important roles in many biological processes in which pharmaceutical intervention may be useful. High level ***expression*** and native purification of GPCRs are important steps in the biochemical and structural characterization of these molecules. Here, we describe enhanced ***mammalian*** ***cell*** ***expression*** and purification of a ***codon*** - ***optimized*** variant of the chemokine receptor CCR5, a GPCR that plays a central role in the entry of the ***human*** immunodeficiency virus-1 (HIV-1) into immune cells. CCR5 could be solubilized in its native state as determined by its ability to be precipitated by 2D7, a conformation-dependent anti-CCR5 antibody, and by the HIV-1 gp120 envelope glycoprotein. The 2D7 antibody recognized immature and mature forms of CCR5 equally, whereas gp120 preferentially recognized the mature form, a result that underscores a role for posttranslational modification of CCR5 in its HIV-1 coreceptor function. The methods described herein contribute to the analysis of CCR5 and are likely to be applicable to many other GPCRs.

L9 ANSWER 7 OF 8 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998019101 MEDLINE
DOCUMENT NUMBER: 98019101 PubMed ID: 9358069
TITLE: ***Codon*** ***optimization*** for high-level ***expression*** of ***human*** erythropoietin (EPO) in ***mammalian*** ***cells*** .
AUTHOR: Kim C H; Oh Y; Lee T H
CORPORATE SOURCE: Biotech Research Institute, LG Chem, Taejeon, South Korea.
SOURCE: GENE, (1997 Oct 15) 199 (1-2) 293-301.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971124

AB Codon bias has been observed in many species. The usage of selective

codons in a given gene is positively correlated with its expression efficiency. As an experimental approach to study codon-usage effects on heterologous gene expression in mammalian cells, we designed two human erythropoietin (EPO) genes, one in which native codons were systematically substituted with codons frequently found in highly expressed human genes and the other with codons prevalent in yeast genes. Relative performances of the re-engineered EPO genes were evaluated with various combinations of promoters and signal leader sequences. Under the comparable set of combinations, mature EPO gene with human high-frequency codons gave a considerably higher level of expression than that with yeast high-frequency codons. However, the levels of EPO expression varied, depending on the alternate combinations. Since the promoters and the signal leader sequences that we used are known to be equally efficient in gene expression, we hypothesized that the varied expression levels were due to the linear sequence between the promoter and the coding gene sequence. To test this possibility, we designed the EPO gene with hybrid codon usage in which the 5'-proximal region of the EPO gene was synthesized with yeast-biased codons and the rest with human-biased codons. This codon-usage hybrid EPO gene substantially enhanced the level of EPO transcripts and proteins up to 2.9-fold and 13.8-fold, respectively, when compared to the level reached by the original counterpart. Our results suggest that the linear sequence between the promoter and the 5'-proximal region of a gene plays an important role in achieving high-level expression in mammalian cells.

L9 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:401730 CAPLUS
DOCUMENT NUMBER: 125:77480
TITLE: Analogs of human keratinocyte growth factor containing modified or deleted cysteine residues
INVENTOR(S): Morris, Charles F.; Kenney, William C.; Chen, Bao-Lu; Hsu, Eric W.
PATENT ASSIGNEE(S): Amgen Inc., USA
SOURCE: PCT Int. Appl., 114 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9611949	A2	19960425	WO 1995-IB971	19951012
WO 9611949	A3	19961212		
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RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2201940	AA	19960425	CA 1995-2201940	19951012
CA 2202075	AA	19960425	CA 1995-2202075	19951012
AU 9537077	A1	19960506	AU 1995-37077	19951012
ZA 9508608	A	19960725	ZA 1995-8608	19951012
EP 785948	A1	19970730	EP 1995-934793	19951012
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
BR 9509329	A	19971014	BR 1995-9329	19951012
CN 1168678	A	19971224	CN 1995-196410	19951012
CN 1169733	A	19980107	CN 1995-196749	19951012
JP 10507080	T2	19980714	JP 1995-513076	19951012
HU 78050	A2	19990728	HU 1999-1071	19951012
HU 78058	A2	19990728	HU 1999-1255	19951012
PL 182888	B1	20020329	PL 1995-319784	19951012
ZA 9508656	A	19960710	ZA 1995-8656	19951013
NO 9701568	A	19970612	NO 1997-1568	19970404
FI 9701536	A	19970609	FI 1997-1536	19970411
AU 9928133	A1	19990722	AU 1999-28133	19990513
AU 745815	B2	20020411		

PRIORITY APPLN. INFO.:		
US 1994-323340	A2	19941013
US 1994-323475	A	19941013
US 1995-487825	A2	19950607

AU 1995-37077 A3 19951012
WO 1995-IB971 W 19951[illegible]

AB Polypeptide analogs of a potent mitogen of non-fibroblast epithelial cell growth, ***human*** keratinocyte growth factor (KGF), are provided having up to the first 24 N-terminal amino acids modified such that cysteine residues corresponding to amino acid positions 1 and 15 of the mature KGF (amino acid positions 32 and 46 of the precursor protein) are deleted or substituted with another amino acid. These modified analogs have substantially improved stability (i.e., reduced problems caused by improper refolding, intermol. disulfide formation, and/or protein aggregation), and are generally purified in a greater yield of sol., correctly folded protein. Moreover, once purified, the analogs are more stable to pH and temp. as compared to the stability of the parent mol. Partial gene synthesis in combination with recombinant DNA methodologies and site-specific mutagenesis were applied to generate the KGF analogs. Escherichia coli ***codon*** - ***optimized*** KGF genes were constructed by PCR amplification of chem. synthesized oligonucleotides, and 3 different ***expression*** vectors (pCFM1156, pCFM1656, and pCFM3102) designed for the prodn. of KGF analogs in E. coli. The plasmid KGF/pDSR.alpha.2 places the KGF cDNA between the SV40 early promoter and the .alpha.-FSH polyadenylation sequences for ***expression*** in mammalian CHO cell culture. In vivo biol. assay of KGF polypeptides produced in E. coli and ***mammalian*** ***cell*** culture demonstrates no apparent differences in bioactivity between any of the analogs and native KGF. The N-terminal 23-residue deletion analog (.DELTA.N23) causes an increase in serum cholesterol in mice in a dose-dependent manner, and also causes a significant decline in blood glucose in an in vivo model of diabetes mellitus.

=> d his

(FILE 'HOME' ENTERED AT 14:33:44 ON 14 AUG 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 14:34:21 ON 14 AUG 2002

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L1          118 S CODON OPTIMIZATION
L2        21610 S HUMAN PROTEIN
L3         5327 S (HUMAN FACTOR IX) OR (HUMAN FACTOR VIII)
L4          359 S CODON (A) OPTIMIZ?
L5            3 S (L1 OR L4) (P) (L2 OR L3)
L6            1 DUPLICATE REMOVE L5 (2 DUPLICATES REMOVED)
L7         9675 S EXPRESSION (P) HUMAN (P) (MAMMALIAN CELL)
L8           32 S L7 (P) (L1 OR L4)
L9            8 DUPLICATE REMOVE L8 (24 DUPLICATES REMOVED)
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=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	41.32	41.53

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-1.24	-1.24

STN INTERNATIONAL LOGOFF AT 14:41:18 ON 14 AUG 2002